COORDINATION OF NITROGENOUS BASES WITH IRON MESO-, PROTO-, AND HEMATOPORPHYRINS

A dissertation submitted to the Board of University Studies, The Johns Hopkins University, in conformity with the requirement for the degree of Doctor of Philosophy

T. HARRISON DAVIES

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Foreword

Part I, which has to do chiefly with theoretical relations, is the joint contribution of Mr. Taylor, Mr. Davies, Mr. Vestling, and myself. Under this joint authorship it was published with the title *Metalloporphyrins. I. Coordination with nitrogenous bases. Theoretical relations* in The Journal of Biological Chemistry, **135,** 543 (1940).

Part II describes experimental results that were obtained by Mr. Davies exclusively. It was published under his authorship with the title *Metalloporphyrins. III. Coordination of nitrogenous bases with iron meso- proto-, and hematoporphyrins* in The Journal of Biological Chemistry, **135,** 597 (1940).

W. Mansfield Clark

COORDINATION OF NITROGENOUS BASES WITH IRON MESO-, PROTO-, AND HEMATOPORPHYRINS

PART I. THEORETICAL

In this series of studies we shall contribute to the systematic description of the behavior of metalloporphyrins in their coordination with nitrogenous bases.

The first method of attack will be the potentiometric. The results will be supplemented by spectrophotometric measurements.

Conant and his students (1, 2) initiated the potentiometric exploration of systems containing heme and a base that coordinates with heme in both the oxidized and reduced states. Such studies have been extended by Barron and Hastings (3) and particularly by Barron (4). Also important contributions to our knowledge of these complex systems have come by way of spectrophotometry, notably at the hands of Hogness, Zscheile, Sidwell, and Barron (5). The assembly of facts thus made available clarifies relations discussed in the earlier, enlightening papers of Anson and Mirsky (6) and Mirsky and Anson (7).

We shall deal with several systems containing not only different bases but metalloporphyrins composed of different metals and different porphyrins; therefore we require an adaptable nomenclature. We shall report the evidence of considerable complexity; therefore we think it wise to outline with care both the presumptions and the restrictions of a theoretical approach. We shall

have to submit our data to various sorts of analysis. Since that of any one sort will be repeated, we shall conserve space by devoting the greater part of this paper to the development of propositions that can be submitted to experimental tests and that may be referred to later by numbers. Exploratory measurements, detailed in the dissertations of Taylor (8), Davies (9), and Vestling (10) are summarized in the following papers by these authors.

Nomenclature

Several of the terms commonly employed in this field stem from those which arose during the early study of the prosthetic group of mammalian blood pigments and are not etymologically good when applied to substances containing metals other than iron and porphyrins other than protoporphyrin IX. Some attempts at reform have left the nomenclature in more or less confusion. We had adopted a simple nomenclature when Drabkin (11) proposed essentially the same system.

A compound formed from a porphyrin and a metal ion is called a metalloporphyrin. The metal and its state may be designated by expressions such as *ferro*, *cobalti*, etc., as in *manganomesoporphyrin IX*. A compound formed from a metalloporphyrin and another substance is designated by prefixing the name of the coordinating substance to that of the metalloporphyrin, as in *nicotine ferriprotoporphyrin*. In running commentaries, where the specific substance is clearly understood, a compound such as cyanide ferrohematoporphyrin may be spoken of as a base metalloporphyrin, *base* here being used in a wide sense.

Our symbols will refer to nitrogenous bases as coordinating substances but we do not wish to imply that only nitrogenous bases are able to coordinate with metalloporphyrins.

It will be assumed that the preparative procedures and certain identifying tests, which confirm what others have described, suffice to identify our porphyrins. If there be occasion to define a porphyrin quite specifically, both the name and the number given in Fischer's (12) system will be used, as in the name *mesoporphyrin IX*.

Scope and General Definition of Problems

Experience in this laboratory has confirmed our guess that some of the general features of these systems will be revealed by

modifying properties pertaining to one or another class of the components. Accordingly, porphyrin, metal, and coordinating base have been changed independently.

What was not foreseen is that when components are modified each of the systems so far studied has some property that restricts severely the range of experimental study. Accordingly the data require some piecing together if one is to obtain a general formulation of the equilibria and a tentative picture of the coordination compounds that are involved. In attempting this we find it essential to have in view the types of reaction that are to be met and equations formulating those under special study. Indeed, both the older data and much of that gathered in our first experiments are inadequate, because there were lacking those formulations that could have indicated critical relations. Also, such criticism of our final conclusions as may be made should involve a scrutiny of the basic assumptions.

The phenomena observed to date show that the following types of reaction occur in systems containing a metalloporphyrin and a coordinating base. The list is restricted to reactions that are pertinent to the immediate subject. As occasion demands, a more detailed formulation will be constructed with the accumulating evidence and a few postulates, and this will serve to account for the ionic charges not mentioned in the following primitive formulations.

1. Reversible association between porphyrin and reduced metal ion.

Example—Ferrous ion + protoporphyrin $\rightleftharpoons$ ferroprotoporphyrin

It will be recognized that this type of reaction, studied by Fischer, Treibs, and Zeile (13), is involved in the preparation of porphyrins from metalloporphyrins and of metalloporphyrins from porphyrins and metal ions. Except under the special conditions which Vestling (10) describes in his study of preparative methods, it will be assumed that the equilibrium state of this reaction is far "to the right." This assumption seems to be generally accepted for the alkaline water, and water-alcohol solutions such as were used in our studies and accordingly we have not examined it with the care that is desired ultimately.

That metal ions combine with porphyrins containing no acidic group other than $\rangle$NH is the most convincing of the evidences

that the predominating species of the metalloporphyrins having carboxyl groups are not normal salts of the carboxylic acids. Other evidence has confirmed the belief that they are coordination compounds involving the pyrryl nitrogens. This will be so far the tacit assumption that our formulations will provide for only one sort of metalloporphyrin, which we have already called a "coordination compound."

2. Reversible oxidation and reduction of the metalloporphyrin, if the metal component be of appropriate type.

Example— Cobaltimesoporphyrin + electron $\rightleftharpoons$ cobaltomesoporphyrin

It seems unnecessary to review the evidence that there is a preferential response to oxidizing and reducing agents, and that when these agents are suitably apportioned the first response is determined by the state of the metal component and that groups characteristic of the porphyrin alone are but slightly affected, if at all. Since each of the metals that we have used is such that 1 equivalent is concerned in the reduction of the quantity of metalloporphyrin containing a gm. atom of metal, formulations will be simplified by retaining this specification.

3. Reversible dissociation of hydrions (or hydroxyl ions).

Since porphyrin, metalloporphyrin, coordinating base, and base metalloporphyrin may have distinctive, or, in some cases, common groups with different acidic dissociation constants, there are numerous theoretical possibilities for the integration of the free energies of acid ionization and of hydrion dilution with the free energy of the ideal oxidation-reduction process. Actually, of course, all may be integrated in what may be called a continuum (Clark (14)). We may simplify matters greatly by anticipating the evidence that under the limited conditions of the measurements the detected, dissociable hydrions (or hydroxyl ions) pertain to the coordination center rather than to groups characteristic of the porphyrin alone or of the coordinating base alone. Ionizations pertaining to the latter two sorts of groups have to be considered under other circumstances. For the most part the range of pH used in the experimental studies was such that the carboxyl groups of the porphyrin can be *assumed* to be ionized.

4. Reversible association between oxidized metalloporphyrin and a nitrogenous base (including cyanide ion) and between reduced metalloporphyrin and the same base.

Examples—

Manganomesoporphyrin + r pyridine $\rightleftharpoons$ pyridine$_r$ manganomesoporphyrin

Manganimesoporphyrin + q pyridine $\rightleftharpoons$ pyridine$_q$ manganimesoporphyrin

It is the difference between the dissociation constants of the oxidized and reduced base metalloporphyrins that makes possible the potentiometric evaluation of these constants. This is the unique feature of the following papers the mathematical introduction of which is given in this paper.

5. Formation of polymers of metalloporphyrin or of a base metalloporphyrin.

On the one hand there will be given evidence of the existence of dimers in certain instances. On the other hand this calls for equations that are very complex and some of them are not amenable to the approximations that serve as guides to the experimental study of the simpler cases. None the less, when occasion demands, the simpler cases can serve as guides to the treatment of the complex.

For the study of a system having even a part of the possible complexity suggested by this compilation, spectrophotometry has one apparent, but not always real, advantage over potentiometry. The latter requires the presence of the oxidized and the reduced components, greatly increasing the number of components; yet electrode measurements provide certain basic data by means of which the free energy changes may be aligned with those of other oxidation-reduction systems. We need not foreshow the importance of this. What may be emphasized now is that each method has distinct limitations.

In order that the part contributed by each reaction to the free energy change of the continuum may be expressed in a manner that helps our understanding of these complex systems, we should have equations relating the experimentally determinable quantities.

A general equation would be unwieldy were it to describe the electrode potential as a function of the activities of all the components that are implied in this list of reactions. Therefore we had best break down the continuum of actually integrated processes and describe the parts by a plexus of equations. Happily, the control of hydrogen ion activities by means of buffer solutions permits us to eliminate consideration of the part played by hydrogen ion activity while developing other features and to deal

with the "pH effect" separately. Type equations for acid-base equilibria need no repetition.

Equations for Association

There remain for particular consideration the equilibria involving the associations between a nitrogenous base and a metalloporphyrin in the reduced and oxidized states. These are unique. Equations for a type case were first developed by Clark, Taylor, Davies, and Lewis (15) from the elementary equation of Conant and Tongberg (2). Only so much of the development will be given here as is necessary to outline the subject and to provide those typical equations that guide experiment. More detail will be found in the dissertations of Taylor (8), Davies (9), and Vestling (10).

Assume a metalloporphyrin that is subjected to oxidation-reduction in the sense

$$\mathbf{O}_n + ne \rightleftharpoons \mathrm{p}\mathbf{R}_m$$

where $\mathbf{O}_n$ represents the oxidant in the fixed state of aggregation designated by n, which is also the number of electrons, e, required for the reduction, and $\mathbf{R}_m$ represents the reductant in the fixed state of aggregation designated by m. If n be greater than 1, we shall now assume no intermediate state of reduction, although it will be important to reconsider this limitation should evidence make it necessary.

$$\mathrm{p}m = n \tag{1}$$

Let both species be under experimental conditions developing no fact that demands consideration of their acid ionizations or ionic charges, as would be necessary to "balance" the "equation" on one side of which appear n electrons. The electrode equation involving accepted conventions[1] is Equation 2.

$$E_h = E_0 + \frac{RT}{nF} \ln \frac{(\mathbf{O}_n)}{(\mathbf{R}_m)^{\mathrm{p}}} \tag{2}$$

Let a nitrogenous base, B, be added to the solution and there coordinate independently with the oxidized and the reduced

[1] See Clark (16).

metalloporphyrin to form the compounds defined by the following relations.

$$O_n + qB \rightleftharpoons O_n B_q$$

$$R_m + rB \rightleftharpoons R_m B_r$$

With brackets to indicate molar concentrations, parentheses to indicate activities, and γ to indicate activity coefficients we may state the equilibrium equations in two forms:

$$\frac{(O_n)(B)^q}{(O_n B_q)} = K_O \qquad (3, a)$$

$$\frac{[O_n][B]^q}{[O_n B_q]} = \frac{\gamma_{O_n B_q}}{\lambda_{O_n}\gamma_B{}^q} K_O = K'_O \qquad (3, b)$$

$$\frac{(R_m)(B)^r}{(R_m B_r)} = K_R \qquad (4, a)$$

$$\frac{[R_m][B]^r}{[R_m B_r]} = \frac{\gamma_{R_m B_r}}{\gamma_{R_m}\gamma_B{}^r} K_R = K'_R \qquad (4, b)$$

An important assumption is that there is no change in the state of aggregation when metalloporphyrin is converted to base metalloporphyrin.

The assumptions noted will deserve strict attention when the precision of measurement and control of all factors reach artistic adjustment to theoretical requirements. Some will receive further, qualitative treatment later.

Define the following sums

$$S_o = n[O_n] + n[O_n B_q] \qquad (5)$$

$$S_r = m[R_m] + m[R_m B_r] \qquad (6)$$

With the equations at hand derive Equation 7, *a*.

$$E_h = E_0 + \frac{RT}{nF}\ln\frac{S_o}{S_r{}^p} + \frac{RT}{nF}\ln\frac{m^p}{n} + \frac{RT}{nF}\ln\frac{K'_O}{(K'_R)^p}$$

$$+ \frac{RT}{nF}\ln\frac{(K'_R + [B]^r)^p}{K'_O + [B]^q} + \frac{RT}{nF}\ln\frac{\gamma_{On}}{\gamma_{Rm}{}^p} \qquad (7, a)$$

If the conditions are such as to permit no serious change in the values of the activity coefficients occurring in Equations 3, *b*

and 4, *b*, we may proceed as if the last term in Equation 7, *a* were constant and K'_O and K'_R were constant. To simplify the typography we shall omit primes of K'_O and K'_R hereafter.

Proposition I—When all conditions are constant except the ratio of S_o to $(S_r)^p$, symmetry of the titration curve is presumptive evidence that p = 1. See also Proposition XII, *b*.

Proposition II—If p = 1, the slope of the titration curve gives the value of *n*.

Practically this is best determined by Proposition XVI. If p = 1, $m = n$. If it be found that $p = m = n = 1$ when sufficient base is present to convert the oxidized and reduced metalloporphyrins practically completely to the respective base metalloporphyrins, it will not necessarily hold that $p = m = n = 1$ for the metalloporphyrin system itself.

We shall continue with the assumption that $p = n = m = 1$ and equal activity coefficients. These assumptions reduce Equation 7, *a* to 7, *b*.

$$E_h = E_0 + 0.0601 \log \frac{S_o}{S_r} + 0.0601 \log \frac{K_O}{K_R} + 0.0601 \log \frac{K_R + [B]^r}{K_O + [B]^q} \quad (7, b)$$

There is included the condition that the temperature is 30°, since the consequences of the numerical coefficient are to be shown graphically.

If [B] be held practically constant, we have Equation 8, to be

$$E_h = E_b + 0.0601 \log \frac{S_o}{S_r} \quad (8)$$

tested by Propositions I and II. Here E_b indicates the potential that would be observed at 50 per cent reduction of the system at constant concentration of free base, which in most instances will obtain practically at constant concentration of total base. If no base be present, $E_b = E'_0$.

Proposition III—If the potential increase with addition of coordinating substance, all other conditions remaining constant, $K_O > K_R$. If the potential change is in the opposite direction, $K_R > K_O$.

We shall confine illustration to the first case.

Proposition IV—If on addition of base, all other conditions remaining constant, the potential approaches a limiting potential, E_2 (see Equation 11), it follows that $q = r$.

The difference $E_2 - E_0$ is determined by the ratio, K_O/K_R, as shown by Equation 11 and as illustrated in Fig. 1. The course of the association curve between E_0 and E_2 is determined by the absolute values of K_O and K_R, and by the concentration of total metalloporphyrin, S.

Particular emphasis may be given to one conclusion that flows from a consideration of these systems and that is implicit in the equations. This is that the change of potential, accompanying the addition of a coordinating base to a fixed mixture of the reduced and oxidized species of the metalloporphyrin, is a measure of the *ratio* of the two dissociation constants. This difference betokens a difference in the free energies of association and where this is large it may need to be considered in the ultimate analysis of the part that complexes of the metalloporphyrins play in catalysis. Furthermore, the fact that other catalysts found in living cells depend upon the association between dissociable prosthetic groups and specific proteins suggests that base metalloporphyrin systems may serve in some degree as models of important general relations. Barron (4) has already pointed to the fact that the potentials of systems containing the same metalloporphyrin can vary considerably with the nature of the base. These specific effects of coordinating bases are referable to the specific values of K_O and K_R. Barron and Hastings (3) and Barron, DeMeio, and Klemperer (17) have applied the shift of potential to the modification of catalytic action.

It is desired to develop Equation 7, b to include the concentration of total coordinating base, S_b, which is directly determinable. The concentration of total base is defined by Equation 9. Other definitions and specifications convenient to the development follow.

$$S_b = [\mathrm{B}] + q[\mathrm{OB}_q] + r[\mathrm{RB}_r] \tag{9}$$

$$S = S_o + S_r \tag{10}$$

$$E_2 = E_0 + 0.0601 \log \frac{K_O}{K_R} \tag{11}$$

$$x = \frac{[O]}{[R]} = \log^{-1} \frac{(E_h - E_0)}{0.0601} \tag{12}$$

$$y = \frac{[OB_q]}{[RB_r]} = \log^{-1} \frac{(E_h - E_2)}{0.0601} \tag{13}$$

$$\alpha = \frac{S_o}{S} \tag{14}$$

$$\beta = \frac{[OB_q] + [RB_r]}{S} \tag{15}$$

With these relations Equation 16 may be derived.

$$E_h = E_0 + 0.0601 \log \frac{S_o}{S_r} + 0.0601 \log \frac{K_O}{K_R} + 0.0601 \log \frac{K_R + (S_b - ZS)^r}{K_O + (S_b - ZS)^q} \tag{16}$$

where, for the restriction that $q = r$

$$Z = \frac{r(y+1)}{(x-y)} [x(1-\alpha) - \alpha] \tag{17}$$

and for the further restrictions that $\alpha = 0.5$ and $r = 2$

$$Z = \frac{(y+1)(x-1)}{(x-y)} \tag{18}$$

For the restriction that $\alpha = 0.5$ but no limitation on the relation of q to r

$$Z = \frac{(qy+r)(x-1)}{(x-y)} \tag{19}$$

Proceed, for the moment, with Equations 16 and 18.

Proposition V—Certain first order, graphical approximations may give useful information, as detailed below.

In the case selected, unique points of the association curve are (*a*) where

$$[B]^2 = K_R \text{ or } 2 \log [B] = \log K_R \tag{20}$$

There $Z = 0.5$, $E_h - E_0 \fallingdotseq 0.0181$, $S_b \fallingdotseq K_R - 0.5S$. The last two relations and the known values of S_b and S make it possible to judge when $S_b \fallingdotseq [B]$. This approximation is least valid at the low parts of the association curve, but, especially when the value of S is low, it will be found frequently that it may be used profit-

ably if not extended below this first unique point. Then S_b may replace [B] in Equation 7, *b* and modified Equation 7, *b* may be used in place of the unwieldy Equation 16; (*b*) the center point of inflection where

$$[B]^4 = K_O K_R \tag{21}$$

There $Z = 1$, $[B] = (S_b - S)$, $\beta = 0.5$, $[RB_r] = [O]$, $[R] = [OB_q]$, and $E_h = (E_0 + E_2)/2$; (*c*) where

$$[B]^2 = K_O \text{ or } 2 \log [B] = \log K_O \tag{22}$$

There $Z = 1.5$ and $E_2 - E_h = 0.0181$.

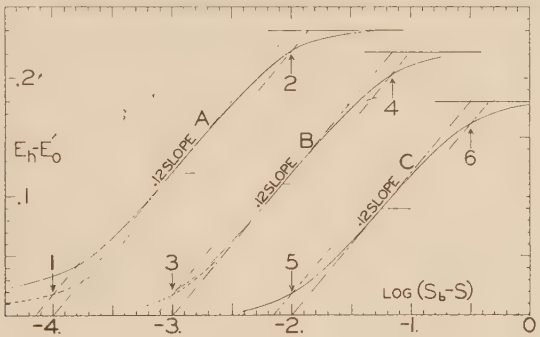

FIG. 1. The solid lines represent the theoretical relation of $E_h - E'_0$ to log $(S_b - S)$ when $q = r = 2$, $0.5S = S_r = S_o = 1 \times 10^{-4}$ M; the broken lines, the same with log $(S_b - 0.5S)$ as abscissa. Curve A, $K_R = 1 \times 10^{-8}$, $0.5 \log K_R = -4.00$ (see Point 1); $K_O = 1 \times 10^{-4}$, $0.5 \log K_O = -2.00$ (see Point 2); $E_2 - E_0 = 0.2400$ (0.06 coefficient). Curve B, $K_R = 1 \times 10^{-6}$, $0.5 \log K_R = -3.00$ (see Point 3); $K_O = 5 \times 10^{-3}$, $0.5 \log K_O = -1.15$ (see Point 4); $E_2 - E_0 = 0.2219$ (0.06 coefficient). Curve C, $K_R = 1 \times 10^{-4}$, $0.5 \log K_R = -2.00$ (see Point 5); $K_O = 1 \times 10^{-1}$, $0.5 \log K_O = -0.50$ (see Point 6); $E_2 - E_0 = 0.1800$ (0.06 coefficient).

For the case under consideration it may be shown that, when $K_O > S_b{}^r > K_R$ at the center point, the slope at the mid-point approaches the uniquely simple, algebraic form (Equation 23):

$$\frac{dE}{d \log(S_b - S)} \doteq (0.0601)r \tag{23}$$

Fig. 1 illustrates a method of graphical analysis based upon the use of these approximations and unique points. Since Z is

unity at the center point, log $(S_b - S)$ is made the abscissa in order that the important orienting diagonal, of slope 0.12, may coincide with the center point. When the ratio of K_O to K_R is as large as any of the values indicated, the diagonal is very close to the tangent through the mid-point and accordingly can be well placed. This diagonal will intersect the line of E'_0 where

$$0.5 \log K_R = \log [B] \tag{24}$$

Now if $[B] \fallingdotseq (S_b - S)$, as it does in Curve C, this point of intersection will be 0.0181 volt below the corresponding point of the association curve, drawn with log $(S_b - S)$ as abscissa. Curves B and A show progressively greater departure from this relation. Yet at the unique point in question $Z = 0.5$, so that, if a supplementary curve be plotted with log $(S_b - 0.5S)$ as abscissa, the unique point falls on the supplementary curve very close to 0.0181 volt above the aforementioned intersection. We shall show that it is not always possible to report a reliable value for E'_0. When deprived of this base-line, one may use the obvious, alternative method shown in Fig. 1; namely, to intersect the appropriate curve with a line parallel to the orienting diagonal and 0.0181 volt above. For the curves shown and on the scale used in Fig. 1 it would be difficult to discern any difference along the upper part of the curves whether the abscissa were made log $(S_b - S)$, log S_b, or the log $(S_b - 1.5S)$ which is demanded for the location of the unique point where

$$0.5 \log K_O = \log [B] \tag{25}$$

If necessary a drawing of larger scale may be used and the principle described for the finding of K_R applied to the estimation of K_O. Fig. 1 illustrates the elements.

Once the orders of magnitude of K_O and K_R, as well as of S, are known, one may estimate the legitimacy of each approximation. In extreme cases it may be necessary to resort to a series of progressively closer approximations but, especially when S is small and the constants large, it suffices to plot with log S_b. The latter is preferable for final description.

It should not go unnoted that previous authors have plotted with S_b as abscissa. The resulting graph obscures those features that are useful in the estimation of K_R and K_O and it may leave

the impression that the potentials at high concentration of base have reached a limit when the evidence thereof is quite inadequate.

Proposition VI—If at and near the center of an association curve K_O is much greater than $[B]^q$ and K_R is much less than $[B]^r$, the value of r will be determined by the maximal slope of the association curve, which is given by Equation 23.

The actual slope need only approximate an integral multiple of 0.0601, since r must be a whole number. The departure will be the greater the smaller the ratio of K_O to K_R. If q be less than r, the effect of combination between base and oxidant will extend over a larger range and may affect the slope of the mid-section.

Comment on Stepwise Association—If association be stepwise, a term such as $K_O + [B]^2$ in Equation 7, b must be replaced by

$$K_{O_1}K_{O_2} + K_{O_1}[B] + [B]^2 \qquad (26)$$

or by a more extended term if the number of steps be greater than two. On statistical grounds stepwise association is more probable than concurrent addition of 2 or more molecules of base and under certain distributions of the energy changes this may be reflected in the equilibrium states. Therefore the theorist will be tempted to provide for this. On the other hand the experimentalist will recognize that the provision of several constants in terms of this kind is not unlike the provision of several constants in those infinite series that are used for the development of empirical equations and becomes devoid of physical significance if his method be not delicate enough to detect the individual values. But be it noted that, as [B] varies, the value of the sum in Equation 26 runs from that of the first term to that of the last. Accordingly, for the purpose of defining the gross contour of an association curve, Equation 26 may be reduced to the corresponding term in Equation 7, b.

The further the departure between the values of the first and second dissociation constants, the more will be the weight of a $K_1[B]$ term, so that experimental identification may become possible. When it is, the equations may be enlarged appropriately.

A Note on Polymers—The potentiometric method can reveal

the presence of polymers. A particular case was described by Stiehler and Clark (18). Barron (4) and Davies (9) show that the components of certain metalloporphyrin systems are dimers. The equations here given are predicated on the assumption that there is no *change* in state of aggregation and where the symbols that have been used imply monomeric components the equations need only slight modification to represent the case in which all metalloporphyrins are dimeric. The situation is entirely different when there is a *change* in state of aggregation accompanying association with a base. Because of the much greater complexity

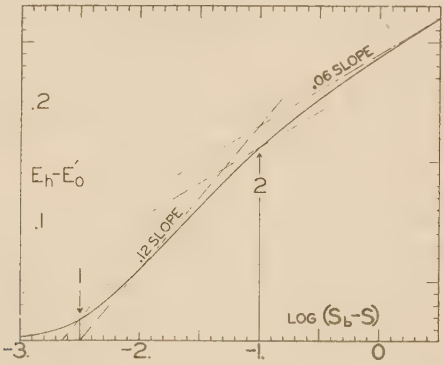

FIG. 2. Theoretical curve. Case of $q = 1$, $r = 2$, $K_R = 1 \times 10^{-5}$, $K_O = 1 \times 10^{-1}$. Point 1, $0.5 \log K_R = \log (S_b - S)$; Point 2, $\log K_O = \log (S_b - S)$.

of such equations we think it wise to postpone their treatment until necessity demands it.

Cases in Which $q < r$ —For such cases Propositions I, II, III, and VI will hold, while IV and V need modification. Fig. 2 illustrates a theoretical case and a method of graphical analysis so similar to that already described as to require no further comment. Fig. 2 will also serve to illustrate some of the propositions that follow.

Proposition VII—If $q < r$, the maintenance of the slope r (0.0601) at high values of [B] should indicate that K_o is infinite, and $q = 0$ (no association of oxidized metalloporphyrin and base).

Proposition VIII—A decline of the slope on further addition of base indicates that K_O is finite and $q > 0$.

On still further addition of base the term 0.0601 log $(K_R + [B]^r)/(K_o + [B]^q)$ approaches 0.0601 log $[B]^r/[B]^q$ or 0.0601 log $(S_b)^{r-q}$ (see Fig. 2). Whence:

Proposition IX—If, at very high concentration of coordinating base, the slope of the association curve approaches a constant value, the slope is given by Equation 27 from which is determined the value of $r - q$.

$$\frac{\Delta E_h}{\Delta \log S_b} = 0.0601(r - q) \qquad (27)$$

Participation of Hydrions—To avoid unnecessary complexity we have followed the policy of breaking down the continuum of actually integrated processes and up to this point have left out of consideration the participation of hydrogen (or hydroxyl) ions. That this is a practical policy flows from the established fact that, if a set of measurements be made in buffer solutions at constant pH, the components of the buffer system will absorb or supply the hydrogen ions liberated or taken up in the reactions hitherto considered and no experimental evidence of their participation will appear, provided, of course, that the buffer system be in great excess. By the same token, a shift in the pH value of the buffer system will alter the ease with which hydrogen ions are made available for the completion of the reaction and the effect will appear in the magnitude of the over-all free energy change, the intensity factor of which is measured by the electrode method.

Since these principles have been developed in previous studies (19), it seems unnecessary to embody them in equations now being used to outline general features, although it will be essential to do so in dealing with specific, actual cases. But there is one aspect that should be given particular emphasis lest the policy now being followed become grievously deceptive.

Prior to potentiometric studies by Conant (1, 2) and Barron (4) it was largely a matter of artistry to assign to ferriprotoporphyrin in alkaline solution a hydroxyl ion, or a species formula that we shall represent by $O\overline{OH}$. Now that potentiometric measurements have shown hydrogen ions or hydroxyl ions to be involved in the equilibria obtaining among some of the systems, we must contemplate the possibility that there is a competition between hydroxyl ions and coordinating base molecules for posi-

tion at the coordination center. To take care of this in the simplest of several possible formulations let us postulate that in the coordination between base and oxidized metalloporphyrin the base coordinates only with the species O, leaving applicable Equation 3, *b* but that, in addition, there obtains the equilibrium represented in Equation 3, *c*.

$$\frac{[\mathrm{OOH}][\mathrm{H^+}]}{[\mathrm{O}]} = k[\mathrm{H_2O}] = K_h \qquad (3,\ c)$$

By following the usual procedure with the inclusion of Equation 3, *c* one arrives at an "electrode equation" comparable with Equation 7, *b* and differing therefrom only in the last term. This term when written in a form suited to the immediate discussion is

$$0.0601 \log \frac{K_R + [\mathrm{B}]^r}{\frac{K_O}{[\mathrm{H^+}]}([\mathrm{H^+}] + K_h) + [\mathrm{B}]^q} \qquad (28)$$

The point to be emphasized is that when association experiments are run at *constant* hydrogen ion concentration the constant called K_O in the foregoing discussion should be in reality $(K_O/[\mathrm{H^+}])([\mathrm{H^+}] + K_h)$. In other words what has been called the dissociation constant of the base metalloporphyrin in oxidized form is an "apparent constant" the value of which is a function of $[\mathrm{H^+}]$.

The general significance of this warning is not dependent upon the particular postulate used above—a postulate which we shall show is too simple.

Proposition X—Previous theoretical and experimental studies (19, 20) have shown that, when the relation between E_h and pH, other conditions being constant, is

$$\frac{-\Delta E_h}{\Delta\ \mathrm{pH}} = 0.0601 \text{ (at } 30°) \qquad (29)$$

the reductant must possess 1 more hydrogen ion (or 1 less hydroxyl ion) than the oxidant for each electron necessary to convert oxidant to reductant.

Evidence will be presented in the experimental papers that the pH effects have to do with the coordination shell so that we may now say that the above relation will demonstrate the reduced

coordination center to possess 1 more hydrogen ion or 1 less hydroxyl ion per metal atom.

Proposition XI—On increase of pH, if the *reductant* lose a equivalents of protons per mole, the value of $-dE_h/d$ pH will decrease by $a(0.0601)/n$ (at 30°); while if the *oxidant* lose a equivalents of protons per mole the value of $-dE_h/d$ pH will increase by $a(0.0601)/n$. Loss of a proton is equivalent to gain of hydroxyl ion. See references (19) and (20) for details on this proposition.

An example will be shown of a change from $-\Delta E_h/\Delta$ pH = 0.0601, for the higher values of pH, to $-\Delta E_h/\Delta$ pH = 0.0 for the lower values of pH. Whatever the structural interpretation may be, this experimental fact demands the *equivalent* of the loss of hydroxyl ion from the oxidant at the lower pH values. Also the mid-point of the transition between the two slopes gives the so called pK value.

Comment on Activities—There remain for consideration troublesome questions arising from changes in activity coefficients that have been neglected up to this point. A rigid treatment of this subject, involving so complicated a set of components, would require a length out of proportion to the scope of the experiments to be reported. Therefore, we may reduce our considerations to the following brief summary of our policy regarding the least certain of the several experimental aspects of this subject.

Simultaneous control of all factors but the one under immediate investigation is not rigidly attained in actual experiments. The greater uncertainties lie in the interpretation of data purporting to yield "pH" values and in corrections for those changes of pH and other factors that attend the modification of the medium. To provide a basis for the discussion of the first it seems advisable to outline here the experimental procedure.

pH Control—In many cases the concentration of added base, such as pyridine or nicotine, is so large as to make impracticable the use of the pH numbers of the solutions containing only the buffer salts. Furthermore, such substances interfere with the proper operation of the hydrogen electrode in the solutions used. Accordingly the glass electrode was employed. The type introduced by MacInnes and Belcher (21) was operated in a shielded, constant temperature, air bath. A Compton quadrant electrome-

ter was used for null point instrument in conjunction with a Leeds and Northrup type K potentiometer. A very close approach to the theoretical change of potential with pH was established with acid and neutral buffers that had been measured with the hydrogen electrode. The glass membrane had to be used with alkaline solutions with which there are well known difficulties. The use of potassium in place of sodium in the buffers materially reduced drifts and deviations. Since the membrane of Corning No. 015 glass behaved normally with mildly acidic buffers, if previous contact with alkaline buffers had not been prolonged, it was assumed that its initial response in alkaline solution was the more reliable. Accordingly rapid measurements were made of the tested solution and of buffer solutions of nearly the same pH numbers, as determined by the hydrogen electrode, and interpolations were made. By this procedure there were found orderly relations between the potentials of the cell with glass membrane and the amounts of alkali added to any one of the various buffer solutions and also orderly relations between the potentials and the amounts of pyridine or alcohol added to aqueous buffers.

If we accept a potential so measured as indicative of a pH number, that number appears to be referred to the standard for aqueous solutions and as if measured with the hydrogen electrode, since the latter remained the means of standardizing the compared solutions. Actually the higher concentrations of pyridine, or nicotine, which in some cases went as high as 3 M, may seriously impair the ordinary interpretation of "pH" and of the standard methods of its computation. Nevertheless, if any one set of data be restricted to solutions having grossly the same composition and if it then be found, on making a "pH" change the major variable, that there is established a close approach to the pH effect predicted by the simplified equations, the following conclusion is drawn. While strict standardization of pH numbers is precluded, the experimental satisfaction of the predicted pH effect in terms of *relative* pH changes establishes the *nature* of a part of the equilibrium. This, rather than precisely referred values, we consider to be of first order importance.

The greater uncertainties are in the experiments purporting to show the course of association. Here occur the largest of the uncontrolled changes of the medium. What we have been forced

to assume are the following. (*a*) The shift of pH accompanying the addition of neutral pyridine, nicotine, etc., is given by glass electrode measurements and can be applied by use of the established pH effect so as to bring all points on the association curve to the basis of constant "pH" on the arbitrary scale. (*b*) The shift of the activity coefficients is *assumed* to be negligible while coordinating base is increased. Here we may retain the same order of magnitude of the ionic strength but the dielectric constant will change.

APPLICATIONS OF SPECTROPHOTOMETRY[2]

Granting that obvious prerequisites for spectrophotometry are met and that *only two* species containing metalloporphyrin absorb at a suitably chosen wave-length, we may use the following relations.

Assume the process

$$aM_m + bA \rightleftharpoons cM_nA_e \tag{30}$$

where A represents either (*a*) base coordinating with M_m at constant pH to form M_nA_e or (*b*) hydrion adding to M_m to form M_nA_e at constant concentration of base. In case (*b*), M may be either metalloporphyrin or base metalloporphyrin. Relation 30 is stated in this general form for economy of space and to avoid needless repetition for the several specific cases. The restriction to two absorbing species places a severe limitation upon the following relations. Relief from this restriction will be noted in a special, experimental case.

Implied in Relation 30 are that:

$$am = cn \tag{31}$$

$$b = ce \tag{32}$$

$$\frac{[M_m]^a[A]^b}{[M_nA_e]^c} = K \tag{33}$$

Define

$$S = m[M_m] + n[M_nA_e] \tag{34}$$

$$\epsilon' = -\log T \tag{35}$$

[2] See also Hogness *et al.* (5).

where T is the transmittance for any mixture of absorbing species at a fixed value of S and given length of absorbing column.[3]

$$\epsilon'_1 = -\log T_{Mm} \tag{36}$$

where T_{Mm} is the limiting value of T where $S = m[M_m]$.

$$\epsilon'_2 = -\log T_{MnAe} \tag{37}$$

where T_{MnAe} is the limiting value of T when $S = n[M_nA_e]$. By application of the rule of the additive property of log transmittances one obtains

$$\frac{[M_m]^a}{[M_nA_e]^c} = \frac{K}{[A]^b} = S^{a-c}n^cm^{-a}(\epsilon'_1 - \epsilon'_2)^{c-a}\frac{(\epsilon'_2 - \epsilon')^a}{(\epsilon' - \epsilon'_1)^c} \tag{38}$$

Proposition XII—(*a*) Symmetry of the curve relating ϵ' to log $[A]$ is presumptive evidence that $a = c$, whence $m = n$.

(*b*) Absence of a "dilution effect" involving S^{a-c} is further evidence. When $a = c$ and $m = n$, Equation 38 reduces to Equation 41 with appropriate interpretations of b and K.

If S is constant, the tangent to the curve at the mid-point, where $\epsilon' - \epsilon'_2 = \epsilon'_1 - \epsilon'$, will be given by

$$\frac{d\log[A]}{d\epsilon'} = \frac{-0.8686}{(\epsilon'_1 - \epsilon'_2)}\left(\frac{a+c}{b}\right) \tag{39}$$

Practically the use of this tangent may not be very certain, but to illustrate uses, consider the following possible cases.

Case 1. $M + 2A \rightleftarrows MA_2$; $a = 1$, $b = 2$, $c = 1$
" 2. $2M + 3A \rightleftarrows M_2A_3$; " = 2, " = 3, " = 1
" 3. $M_2 + 2A \rightleftarrows M_2A_2$; " = 1, " = 2, " = 1

All of these give the same value of $(a + c)/b$. Yet Cases 2 and 3 would require 2 equivalents for the oxidation-reduction of the *base* metalloporphyrin. Therefore, if a symmetrical, potentiometric curve involving 1 equivalent were to be found, Case 1 could be presumed to apply and the slope now under discussion would indicate that $b = 2$. An additional and better test for

[3] The symbol ϵ' is introduced for economy of space in the more complicated equations. With apologies to the reader we ask that he carefully distinguish ϵ' from ϵ (see Table I).

the value of b is discussed in the following section. Be it noted that the spectrophotometric test alone would not distinguish Case 1 from Case 3.

Proposition XIII—If $a = c$ the center of the symmetrical curve relating ϵ' to log $[A]$ is where $\epsilon'_2 - \epsilon' = \epsilon' - \epsilon'_1$ and at this point

$$\log K = b \log [A] \tag{40}$$

RECTIFICATION OF CURVES

The general method of Reed and Berkson (22) is used.

Rectification of Spectrophotometric Curves. Relation of $-$ *Log* T (*or* ϵ') *to Log A*—This is limited to the following modification of Equation 38.

$$\frac{[M_m]}{[M_m A_b]} = \frac{K}{[A]^b} = \frac{\epsilon'_2 - \epsilon'}{\epsilon' - \epsilon'_1} \tag{41}$$

Choose an orienting pair of values called ϵ'_0 and log $[A]_0$ and define

$$b \log [A] - b \log [A]_0 = p \tag{42}$$

$$\log K - b \log [A]_0 = -\log G \tag{43}$$

where $p = 0$, when $\epsilon' = \epsilon'_0$. Derive

$$G\, 10^p = \frac{\epsilon' - \epsilon'_1}{\epsilon'_2 - \epsilon'} \tag{44}$$

$$G = \frac{\epsilon'_0 - \epsilon'_1}{\epsilon'_2 - \epsilon'_0} \tag{45}$$

Whence

$$\epsilon' = \epsilon'_1 + (G + 1)\frac{(\epsilon'_0 - \epsilon')10^p}{1 - 10^p} \tag{46}$$

Proposition XIV—If the assumed value of b fits the actual case, a chart of ϵ' against $((\epsilon'_0 - \epsilon')10^p)/(1 - 10^p)$ should give a straight line the properties of which determine K, ϵ'_1, and ϵ'_2.

The slope is $(G + 1)$, whence K is found by Equation 43. The intersection with the ϵ' axis gives ϵ'_1. Thence ϵ'_2 is found by Equation 45. If desired, the equation can be recast to yield ϵ'_2 by intersection.

Rectification of Potentiometric Association Curves Relating E_h to Log S_b—Let Equation 7, b be solved for $[B]^q$ under the condition that $q = r$. Assume $[B] \doteq S_b$. Define

$$\log^{-1} \frac{E_h}{0.0601} = u \tag{47}$$

$$\log^{-1} \frac{E_2}{0.0601} = J \tag{48}$$

$$q \log S_b - q \log S_{bo} = p \tag{49}$$

$$-q \log S_{bo} = \log C \tag{50}$$

The chosen pair of orienting data are u_o and S_{bo} where $p = 0$. Derive

$$u = J + (CK_O + 1) \frac{u_o - u}{10^p - 1} \tag{51}$$

Proposition XV —When previous knowledge justifies the use of the approximation carried into Equation 51, the slope of the straight line relating u to $(u_o - u)/(10^p - 1)$ is $(CK_O + 1)$ which yields the value of K_O by use of Equation 50 and the intersection with the u axis yields E_2 by Equation 48.

Similar relations leading to the evaluation of K_R and E'_0 could be obtained by use of $u' = \log^{-1} (-E_h/0.0601)$ and $J' = \log^{-1} (-E_0/0.0601)$.

The use of this method of rectification is tricky for the following reasons.

If the orienting values of u_o and $\log S_{bo}$ be taken from near the center of the association curve where the effects of K_O and K_R are small, the slope of the rectified line approaches unity and is of no value in the determination of either constant. The approximation carried into the equation, namely $S_b \doteq [B]$, is least valid at the lower part of the association curve. Therefore we hesitate to use this method for evaluating K_R. The following simpler rectification may be applicable in rare cases. Assume $q = r$, $S_o = S_r$, $S_b = [B]$, $K_O >> [B]^q$. Whence $E_h = E'_0 + 0.0601 \log (K_R + S_b{}^r)/K_R$. Define $u = \log^{-1} (E_h/0.0601)$ and $E_0 = 0.0601 \log W$. Whence $u = W + (W/K_R) S_b{}^r$. Thus a plot of u against $S_b{}^r$ should approximate a straight line the proper-

ties of which allow estimate of K_R but only a crude estimate of E'_0.

Rectification of Titration Curves

Reed and Berkson's method as applied to this case has been discussed by Clark and Perkins (23). We shall use the symbols defined by the latter authors as follows:

$y \equiv$ number of ml. of specified reducing agent required to reach the potential E_h

$d' \equiv$ number of ml. of the reducing agent added before the main system is attacked

$A \equiv$ number of ml. of the reducing agent required to carry the main component from 0 to 100 per cent reduction

E_n and y_n are chosen orienting values of E_h and y respectively

$$P = \frac{E_h + E_n}{1/n(0.0601)} \tag{52}$$

$$E'_0 + E_n = -\frac{0.0601}{n} \log C \tag{53}$$

Whence

$$y = (C + 1)\frac{y_n + y10^P}{1 - 10^P} - dC \tag{54}$$

Proposition XVI—The properties of a titration curve are determined by the properties of a straight line the coordinates of which are y and $(y_n + y10^P)/(1 - 10^P)$ according to Equation 54.

The mid-point potential, E'_0, is determined by the slope $(C + 1)$ and thence all other characteristics of the titration curve are evaluated as described by Clark and Perkins.

The method is predicated on the applicability of Equation 8 and in particular upon an integral value of n. It is important to note the following. It is possible that the method may yield such numerical values of the constants that a theoretical, sigmoid curve, constructed with these values, may be shifted to a position of best correspondence with an actual titration curve that is slightly asymmetric. To be sure, only a slight asymmetry would go undetected; nevertheless there remains danger of overlooking something. For the present we may say that we know

of no better criterion of the validity of deduced values to be reported unless it be so extreme a precision of measurement and so rigid a control of variables as to make the mathematical analysis by the method of Reed and Berkson superfluous. All titration data are analyzed by this method.

SUMMARY

A nomenclature for metalloporphyrins and their coordination compounds is proposed.

The reactions that may take place in systems containing metalloporphyrins and coordinating bases are typed to define the restrictions of the immediate study.

There are developed theoretical equations relating the electrode potential, the concentration of total metalloporphyrin, the total base that coordinates independently with reduced and oxidized metalloporphyrin, and the ratio of concentrations of oxidized and reduced metalloporphyrin.

A brief review is given of the principles governing the study of the part played by the acid-base equilibria and of experimental difficulties in the application of these principles to the cases at hand.

Approximate forms of the equations are developed to show graphical methods of determining the constants that are descriptive of the dissociation of oxidized and reduced coordination complexes and to show methods of determining the numbers of molecules of coordinating substance that associate on the one hand with reduced metalloporphyrin and on the other hand with oxidized metalloporphyrin.

Equations are given for the use of spectrophotometric data in determining the various constants that define the equilibria of the systems.

Equations are given for rectification of curves.

Sixteen propositions and corollaries thereto are offered as subject to the experimental tests applied in the subsequent papers.

TABLE I

List of More Important Symbols

[B] = molar concentration of free, uncombined, coordinating base
E_h = electrode potential referred to the hydrogen standard
E_b = E_h at constant S_b, other conditions being specified in the text
E'_0 = E_h at 50% reduction of metalloporphyrin, $S_b = 0$, other conditions being specified in the text
e = 1 equivalent of electrons
F = the faraday
K_O = dissociation constant of oxidized base metalloporphyrin
K_R = dissociation constant of reduced base metalloporphyrin. Note, special dissociation constants are defined as needed
n = (1) number of equivalents required to reduce 1 mole of oxidant; (2) number of gm. atoms of metal per mole of oxidant
$[\mathbf{O}_n]$ = molar concentration of oxidized metalloporphyrin containing n gm. atoms of metal ion per mole
[OOH] = molar concentration of oxidized metalloporphyrin associated with 1 mole of hydroxyl ions per mole
$[\mathbf{B}_q\mathbf{O}_n]$ = molar concentration of oxidized, base metalloporphyrin containing q moles of base per mole
$\epsilon' = -\log T = \epsilon cl$
p = ratio of gm. atoms of metal per mole of oxidant to gm. atoms of metal per mole of reductant
q = number of moles of base associating with 1 mole of oxidized metalloporphyrin
r = number of moles of base associating with 1 mole of reduced metalloporphyrin
$[\mathbf{R}_m]$ = molar concentration of reduced metalloporphyrin
$[\mathbf{B}_r\mathbf{R}_m]$ = molar concentration of base metalloporphyrin containing r moles of base per mole
R = gas constant
$S = S_o + S_r$
S_o = sum of apparent molar concentrations of oxidized metalloporphyrin and oxidized base metalloporphyrins
S_r = sum of apparent molar concentration of reduced metalloporphyrin and reduced base metalloporphyrins
S_b = sum of apparent molar concentrations of uncombined base and base metalloporphyrins
T = absolute temperature
T = transmittance

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PART II. EXPERIMENTAL

The potentiometric study of heme in coordination with nitrogenous bases, initiated by Conant and his students, has been greatly extended by Barron (1). Barron and Hastings (2) announced a summary of their earlier data when a plan of a more general study of metalloporphyrins was being drawn in this laboratory. They graciously agreed that we should continue with the subject and give special attention to the effects of changing independently the metal and porphyrin components. It was our original intention to repeat some of their work only to profit by their invaluable experience but as there developed from the study of other systems the evidences of unsolved problems, we have felt justified in recovering some of the ground examined by Barron. Data for systems containing iron protoporphyrin IX, such as were obtained by Barron, are now compared with analogous data for systems containing iron mesoporphyrin IX and the corresponding iron hematoporphyrin, but within the restricted limits set by the properties of those systems that are adapted to potentiometric and spectrophotometric study.

Preparations

Preparations of hemin chloride and of mesoporphyrin IX have been described in Paper II of this series (see Taylor (3)).

The first of these materials was, as a matter of course, the form in which we employed iron protoporphyrin IX.

Ferrimesoporphyrin chloride was prepared by the method of Fischer and Stangler (4) from mesoporphyrin hydrochloride.

Hematoporphyrin dimethyl ether was prepared according to the method of Küster *et al.* (5) except in minor detail. It was recrystallized from 90 per cent methyl alcohol, and used in the preparation of iron hematoporphyrin chloride.

Ferrihematoporphyrin chloride proved difficult to prepare in pure state. Recrystallized hematoporphyrin dimethyl ether (1.2 gm.) was dissolved in 70 per cent acetic acid (75 ml.) saturated with sodium chloride. To this solution, held at 90°, was added an acetic acid solution of ferrous acetate until the color changed from red to brown. After further heating at 90° for 10 minutes the solution was cooled. The precipitate was collected and washed with a little methyl alcohol containing hydrochloric acid. The yields of several preparations averaged 0.7 gm. The method did not yield uniform preparations. Several black samples were semicrystalline but proved to be mixtures. Only one preparation, a brown amorphous material, when treated with cyanide gave a titration curve typical of a compound pure with respect to titratable material. In preparation of the metalloporphyrin from the dimethyl ether, the latter presumably should be hydrolyzed before it coordinates with the metal ion. It is conceivable that a portion of the hematoporphyrin dimethyl ether may coordinate, oxidize, precipitate, and thus escape hydrolysis. It might appear better to prepare hematoporphyrin itself and to form the metalloporphyrin directly from this; but in the first place it is difficult to prepare in pure state and in the second place its use in hot acetic acid solutions sufficiently strong to favor the formation of the desired crystals might lead to protoporphyrin (Fischer and Zeile (6)). The latter would affect our potentiometric measurements more seriously than would miscellaneous impurities of other kinds.

The identity of our preparations of ferrihematoporphyrin with those described by Fischer, Küster, and others is reasonably well established by the similarity of the methods of preparation, by the close correspondence between the spectral absorptions, and, in so far as analysis is valuable in such cases, by the agreement between found and calculated iron content.

The three metalloporphyrin preparations were analyzed for iron by the method of Klumpp (7). His procedure was altered in a few details for convenience. The titrations were performed under nitrogen instead of the recommended carbon dioxide. The titanous sulfate solution was standardized against a permanganate solution instead of against a ferric ammonium sulfate solution. The results appear in Table I.

TABLE I

Analyses of Metalloporphyrins for Iron

	Weight of sample	$Ti_2(SO_4)_3$ solution required	Normality of $Ti_2(SO_4)_3$ solution	Fe found	Fe calculated	Formula
	mg.	*ml.*		*per cent*	*per cent*	
Ferriprotoporphyrin chloride	89.6	5.82	2.232×10^{-2}	8.10	8.57	$C_{34}H_{32}O_4N_4FeCl$
	92.9	6.10	2.232×10^{-2}	8.18		
Ferrimesoporphyrin chloride	98.1	6.45	2.232×10^{-2}	8.19	8.52	$C_{34}H_{36}O_4N_4FeCl$
	96.4	6.32	2.232×10^{-2}	8.17		
Ferrihematoporphyrin chloride	43.2	7.88	8.03×10^{-3}	8.14	8.12	$C_{34}H_{36}O_6N_4FeCl$

Description of Results

Series of data have been analyzed in terms of the several propositions set forth in Paper I (8) and for brevity these propositions are referred to here by number.

Potentiometric Measurements

Titrations in Absence of Coordinating Base—

$$O_n + ne \rightleftharpoons pR_m$$

The electrode potentials of mixtures of ferrimesoporphyrin and ferromesoporphyrin exhibit drifts as if reductant were being withdrawn continuously from the field. Since precipitates of the bright red reductants were observable and the entire picture conformed to what Taylor found in his study of the cobalt and manganese mesoporphyrins, it seemed reasonable to attribute

instability of potential to progressive precipitation of the reductant. On the other hand the same electrode behavior was observed with alcohol solutions in which there appeared adequate solubility, and Vestling, working with the water-soluble ferrocoproporphyrin, encountered similar positive drifts. Barron (1) reports success in electrode measurements with iron protoporphyrin but emphasizes the lack of strict reversibility of this system. Since we have not yet succeeded in obtaining a wholly

TABLE II

Cyanide Iron Hematoporphyrin System

Titration of cyanide ferrihematoporphyrin with reduced phthiocol at constant pH and constant cyanide concentration. Demonstration that $n = 1$. NaCN = 1.63×10^{-2} M; total pigment = 1.72×10^{-4} M; phthiocol $\fallingdotseq$ 1.5×10^{-3} N; pH of phosphate buffer = 10.77; $\mu = 0.18$; $d = +0.22$ ml.; temperature = 30°; 100 per cent reduction at $y = 5.3$ ml.

y	$y - d$	Reduction	$0.06011 \times \log \frac{S_r}{S_o}$	E_h	E_b	Deviation from average
ml.	*ml.*	*per cent*		*volt*	*volt*	*volt*
1.0	0.78	15.354	−0.0446	−0.1536	−0.1982	0.0021
1.3	1.08	21.259	−0.0342	−0.1622	−0.1964	0.0003
1.58	1.36	26.771	−0.0263	−0.1699	−0.1962	0.0001
1.88	1.66	32.677	−0.0189	−0.1775	−0.1964	0.0003
2.18	1.96	38.582	−0.0121	−0.1841	−0.1963	0.0002
2.48	2.26	44.488	−0.0054	−0.1906	−0.1960	−0.0001
2.78	2.56	50.393	0.0004	−0.1966	−0.1963	0.0002
3.08	2.86	56.299	0.0067	−0.2030	−0.1963	0.0002
3.38	3.16	62.204	0.0130	−0.2092	−0.1961	0.0000
3.68	3.46	68.110	0.0198	−0.2158	−0.1959	−0.0002
3.98	3.76	74.015	0.0273	−0.2228	−0.1965	−0.0004
4.58	4.36	85.826	0.0470	−0.2490	−0.1920	−0.0041
Average					−0.1961	

reliable set of potentiometric measurements with any one of the metalloporphyrin systems in the *absence* of coordinating bases, we shall not report the measurements made in this very important part of the field. In some instances a fair estimate of the characteristic potentials may be calculated with certain assumptions and, should the accumulating data converge, it may be possible then to see more clearly the reasons for the electrode instability, and to subject these to special experimental tests.

Titrations in Presence of Coordinating Bases—

$$O_nB_q + ne \rightleftharpoons pR_mB_r$$

When certain bases are added to buffered solutions containing ferro- and ferriporphyrins, the electrode potentials become

TABLE III

Pyridine Iron Mesoporphyrin System

Titration of pyridine ferrimesoporphyrin with reduced phthiocol in alcohol-buffer mixture at constant pH and constant pyridine concentration. Demonstration that $n = 1$. Solvent, 47.5 per cent ethanol; pyridine = 4.56×10^{-1} M; total pigment = 1.47×10^{-4} M; phthiocol = 1.6×10^{-3} N; pH of phosphate buffer + ethanol + pyridine = 11.22; $\mu = 0.19$; temperature = 30°; $d = 0$; 100 per cent reduction at $y = 4.64$ ml.

y	Reduction	$0.06011 \times \log \frac{S_r}{S_o}$	E_h	E_b	Deviation from average
ml.	*per cent*		*volt*	*volt*	*volt*
0.2	4.314	−0.0809	−0.0761	−0.1570	0.0006
0.4	8.629	−0.0616	−0.0959	−0.1565	0.0010
0.6	12.944	−0.0498	−0.1075	−0.1573	0.0003
0.9	19.417	−0.0372	−0.1201	−0.1573	0.0003
1.2	25.889	−0.0275	−0.1303	−0.1578	−0.0002
1.5	32.362	−0.0193	−0.1384	−0.1577	−0.0001
1.8	38.834	−0.0119	−0.1458	−0.1578	−0.0002
2.1	45.307	−0.0049	−0.1527	−0.1576	0.0000
2.4	51.779	0.0018	−0.1597	−0.1579	−0.0003
2.7	58.252	0.0087	−0.1668	−0.1581	−0.0005
3.0	64.724	0.0158	−0.1739	−0.1580	−0.0007
3.3	71.197	0.0236	−0.1814	−0.1578	−0.0002
3.6	77.669	0.0325	−0.1902	−0.1577	−0.0001
3.9	84.142	0.0435	−0.1996	(−0.1561)	0.0015
4.2	90.614	0.0592	−0.2111	(−0.1520)	0.0056
4.5	97.08	0.0911	−0.2295	(−0.1384)	0.0192
Average				−0.1576	

remarkably steady, provided attention is given to conditions that maintain both the oxidized and reduced metalloporphyrins in solution. The very low solubility in aqueous solution of ferromesoporphyrin and its base complexes was particularly troublesome and because of it aqueous alcoholic solutions were employed. In the use of nicotine, rapid negative potential drifts

were encountered similar to those noted by Taylor with his metalloporphyrins and nicotine. These were eliminated by the procedure employed by him.

Tables II to VI exhibit fairly representative titration data and make it unnecessary to tabulate the data of all cases. A summary will be given. Analysis of the results by Propositions

TABLE IV

Nicotine Iron Protoporphyrin System

Titration of nicotine ferriprotoporphyrin with reduced phthiocol in alcohol-buffer mixture at constant pH and constant nicotine concentration. Demonstration that $n = 1$. Solvent, 47.5 per cent ethanol; nicotine = 3.53×10^{-1} M; total pigment = 1.44×10^{-4} M; phthiocol $\fallingdotseq 1.59 \times 10^{-3}$ N; pH of borate buffer = 10.232; $\mu = 0.05$; temperature = 30°. 100 per cent reduction at $y = 4.682$ ml.

y	$y - d$	Reduction	$0.06011 \times \log \frac{S_r}{S_o}$	E_h	E_b	Deviation from average
ml.	*ml.*	*per cent*		*volt*	*volt*	*volt*
0.2	0.32	6.663	−0.0689	0.0605	(−0.0084)	−0.0010
0.4	0.52	10.828	−0.0550	0.0476	−0.0074	−0.0000
0.7	0.82	17.076	−0.0413	0.0340	−0.0073	0.0001
1.0	1.12	23.323	−0.0311	0.0239	−0.0072	0.0002
1.3	1.42	29.571	−0.0227	0.0154	−0.0073	0.0001
1.7	1.82	37.900	−0.0129	0.0053	−0.0076	−0.0002
2.1	2.22	46.230	−0.0040	0.0030	−0.0070	0.0004
2.5	2.62	54.560	0.0048	−0.0124	−0.0076	−0.0002
2.9	3.02	62.890	0.0138	−0.0214	−0.0076	−0.0002
3.3	3.42	71.220	0.0237	−0.0314	−0.0077	−0.0003
3.7	3.82	79.550	0.0355	−0.0433	−0.0078	−0.0004
4.1	4.22	87.880	0.0517	−0.0589	−0.0072	0.0002
4.4	4.52	94.127	0.0725	−0.0800	−0.0075	−0.0003
4.6	4.72	98.292	0.1058	−0.1057	(−0.0001)	0.0073
Average					−0.0074	

I, II, and XVI led to objective evaluations of p and n, and also of d, the quantity of reducing agent oxidized by impurities. The correction for the latter is always small and even in the case of the iron hematoporphyrin sample, under suspicion because of preparative difficulties, it demonstrates that the bulk of the material titrated as would a substance supplying a homogeneous

oxidation-reduction system. The correction factors of numerous titrations of the same metalloporphyrin preparations with different bases and with different preparations of the same base varied. Accordingly, we interpret the d value to be a measure of the oxidizing impurity introduced to the solution with the relatively excessive quantities of base.

Comparison of Tables IV and V reveals a remarkable contrast between nicotine iron protoporphyrin in *aqueous* solution and the other cases. While titration data of other systems and even of

TABLE V

Nicotine Iron Protoporphyrin System

Titration of nicotine ferriprotoporphyrin with reduced phthiocol at constant pH and constant nicotine concentration. Demonstration that $n = 2$. Nicotine = 2.9×10^{-1} M; total pigment = 4.76×10^{-4} M; pH of phosphate buffer + nicotine = 10.65; $\mu = 0.16$; $d = 0.80$ ml.; 100 per cent reduction at $y = 8$ ml.; temperature = 30°.

y	$y - d$	Reduction	$0.03006 \times \log \frac{S_r}{S_o}$	E_h	E_b	Deviation from average
ml.	*ml.*	*per cent*		*volt*	*volt*	*volt*
1.0	0.2	2.777	−0.0461	−0.0089	(−0.0550)	−0.0065
1.5	0.7	9.722	−0.0295	−0.0201	(−0.0496)	−0.0011
2.0	1.2	16.666	−0.0209	−0.0273	−0.0483	0.0003
2.5	1.7	23.611	−0.0153	−0.0330	−0.0484	0.0002
3.0	2.2	30.550	−0.0107	−0.0378	−0.0485	0.0000
4.0	3.2	44.444	−0.0029	−0.0455	−0.0485	0.0001
5.0	4.2	58.330	0.0044	−0.0529	−0.0485	0.0000
6.0	5.2	72.222	0.0125	−0.0613	−0.0488	−0.0003
7.0	6.2	86.111	0.0238	−0.0726	−0.0488	−0.0003
Average					−0.0485	

nicotine iron protoporphyrin in *alcoholic* solution give the expected value of 1 for n, repeated titrations of nicotine protoporphyrin in *aqueous* solution give clear evidence that n is 2. Since but 1 equivalent of reducing agent per gm. atom of iron is necessary to effect complete reduction, and since the spectral changes closely resemble those of other systems, it is proper to assume that a ferrous-ferric change is being followed. We must conclude that we have to deal with dimers containing 2 atoms of iron per molecule. That *both* oxidant and reductant are dimers is shown

by the symmetry of the titration curves. The distinction between the conduct of nicotine iron protoporphyrin in aqueous and in aqueous alcoholic solution must be kept in mind in what is to follow.

Effect of pH—The examination of the effect of pH proves more difficult with the systems under consideration than with ordinary oxidation-reduction systems. Frequently, so much coordinating base was added as to render calculation of the change in acid-base

TABLE VI

Pyridine Iron Hematoporphyrin System

Titration of pyridine ferrihematoporphyrin with reduced phthiocol at constant pH and constant pyridine concentration. Demonstration that $n = 1$. Pyridine = 1.13 M; total pigment = 1.11×10^{-4} M; phthiocol = 1.5×10^{-3} N; pH of phosphate buffer + pyridine = 11.75; $\mu = 0.50$; $d = +0.20$ ml.; temperature = 30°; 100 per cent reduction at $y = 3.4$ ml.

y	$y - d$	Reduction	$0.06011 \times \log \frac{S_r}{S_o}$	E_h	E_b	Deviation from average
ml.	*ml.*	*per cent*		*volt*	*volt*	*volt*
0.5	0.3	9.375	−0.0592	−0.0570	−0.1162	−0.0005
0.9	0.7	21.875	−0.0332	−0.0800	−0.1132	0.0025
1.3	1.1	34.375	−0.0169	−0.0976	−0.1145	0.0012
1.6	1.4	43.750	−0.0066	−0.1093	−0.1159	−0.0013
2.0	1.8	56.250	−0.0066	−0.1239	−0.1173	0.0004
2.3	2.1	65.625	0.0169	−0.1348	−0.1179	−0.0022
2.6	2.4	75.000	0.0287	−0.1462	−0.1175	−0.0018
2.9	2.7	84.375	0.0441	−0.1568	−0.1127	0.0029
3.2	3.0	93.750	0.0707	−0.1719	(−0.1012)	0.0062
Average					−0.1157	

properties of the buffer systems uncertain. Further, the effect of the nitrogenous base addition upon the systems could not be measured by the hydrogen electrode, for the latter was found unreliable in the presence of such materials. Recourse was had to the glass electrode, although the high alkalinity of our solutions impaired the accuracy of this device.

The method of operation, the general nature of the results, and the limitations of interpretation placed upon "pH" values have been briefly outlined in Paper I (8).

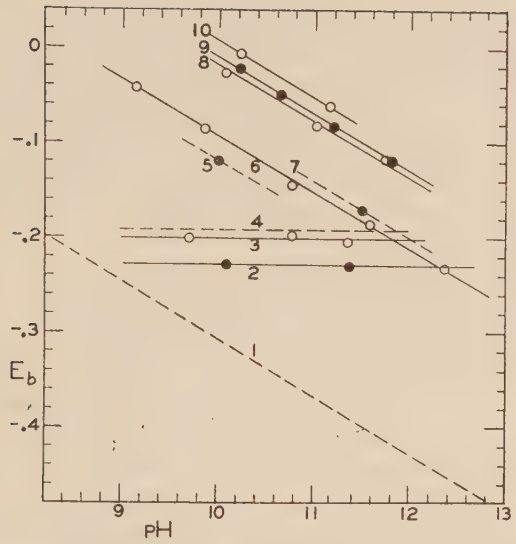


FIG. 1. Relation of E_b to pH. E_b is the potential at 50 per cent reduction and constant concentration of coordinating base. Curve 1, estimated for iron protoporphyrin (see Barron (1)); Curve 2, cyanide iron mesoporphyrin in water solution, total metalloporphyrin 1.25×10^{-4} M, cyanide 8.15×10^{-3} M, $E_b = -0.2291 \pm 0.0005$ volt; Curve 3, cyanide iron hematoporphyrin in water solution, total metalloporphyrin 1.72×10^{-4} M, cyanide 0.022 to 0.0082 M, $E_b = -0.200 \pm 0.004$ volt; Curve 4, cyanide iron protoporphyrin in water solution (see Barron (1)); Curve 5, α-picoline iron hematoporphyrin in water solution, total metalloporphyrin 1.32×10^{-4} M, α-picoline 9.3×10^{-1} M, a single titration at pH 10.05, $\mu = 0.1$, $E_b = -0.1224$ volt; Curve 6, pyridine iron mesoporphyrin in 47.5 per cent ethanol solution, total metalloporphyrin 1.47×10^{-4} M, pyridine 0.456 M, $\mu = 0.05$; Curve 7, nicotine iron mesoporphyrin in 47.5 per cent ethanol solution, total metalloporphyrin 1.47×10^{-4} M, nicotine 2.41×10^{-1} M, a single titration at pH 11.5, $\mu = 0.19$, $E_b = -0.1761$ volt; Curve 8, pyridine iron hematoporphyrin in water solution, total metalloporphyrin 1.1×10^{-4} M, pyridine 1.13 M, $E_b = 0.5856 - 0.0601$ pH, maximum deviation -0.009 volt; Curve 9, nicotine iron protoporphyrin in water solution, $n = 2$, total metalloporphyrin 4.76×10^{-4} M, nicotine 0.29 M, $\mu = 0.2$, $E_b = 0.5909 - 0.0601$ pH, maximum deviation -0.0012 volt; Curve 10, nicotine iron protoporphyrin in 47.5 per cent ethanol solution, total metalloporphyrin 1.44×10^{-4} M, nicotine 0.353 M, $\mu = 0.05$, $E_b = 0.6075 - 0.0601$ pH, maximum deviation 0.0002 volt.

Fig. 1 summarizes the data on the relation of "pH" to the characteristic potentials of the several systems each at constant

concentration of total base, S_b, approximately constant ionic strength, constant temperature (30°), and 50 per cent reduction. Other details are given in the legend of Fig. 1.

Consideration of the contrast between the cyanide compounds, the potentials of which are invariant with pH, and the other systems which exhibit the relation $-\Delta E_h/\Delta \text{ pH} = 0.0601$ will be postponed to the section dealing with a postulated structural basis for the difference.

Potentiometric Measurements of Association—

$$\mathbf{O} + q\mathrm{B} \rightleftharpoons \mathbf{O}\mathrm{B}_q \text{ and } \mathbf{R} + r\mathrm{B} \rightleftharpoons \mathbf{R}\mathrm{B}_r$$

The potentiometric measurements of association were limited in scope: first, by reason of the instability of potential at low base concentration, a situation that approaches the case of a metalloporphyrin in the absence of base and that has been mentioned already; second, by the difficulty of resolving the effect of added base alone when the concentration of base becomes so high as to affect seriously other factors that must be controlled. Nevertheless, significant data were obtained in favorable cases. Diagrams are used to present the more important data, since they lend themselves to the proximate analysis outlined in Paper I of this series.

Fig. 2 gives an excellent example of the obscuring character of an association curve plotted with S_b as abscissa. The theoretical curve at the left has been drawn with constants calculated as described below but it is difficult to see any of the characteristics of the system. On the other hand the curve with log S_b as abscissa is revealing.

The data were submitted to the graphical analysis of Proposition V properly adjusted to the evidence of Table V that $n = 2$ and $p = 1$. Accordingly the proximate placement of the central portion of the curve was made with log $(S_b - 2S)$ as abscissa (case of $Z = 2$). A supplementary curve with log $(S_b - S)$ as abscissa was used to place the lower unique point where $K_R \fallingdotseq [\mathrm{B}]^4$. The final theoretical curve of Fig. 2 has been drawn with log S as abscissa, after slight empirical adjustments of the constants to obtain a good fit. The following deductions are drawn from the *prima facie* evidence by objective methods of analysis.

By Proposition III nicotine coordinates more readily with the

reductant than with the oxidant. Since Table V shows that $n = 2$ and $p = 1$, a proper use of Proposition VI indicates that $r = 4$. The graphical analysis gives $K_R = 1.2 \times 10^{-10}$ and $E'_0 = -0.2570$ (at 50 per cent reduction $E'_0 = -0.2381$). The departure of the maximal slope from 0.12 is greater than would be expected from the first order approximation of the ratio of K_O to K_R were the same number of molecules of nicotine to combine

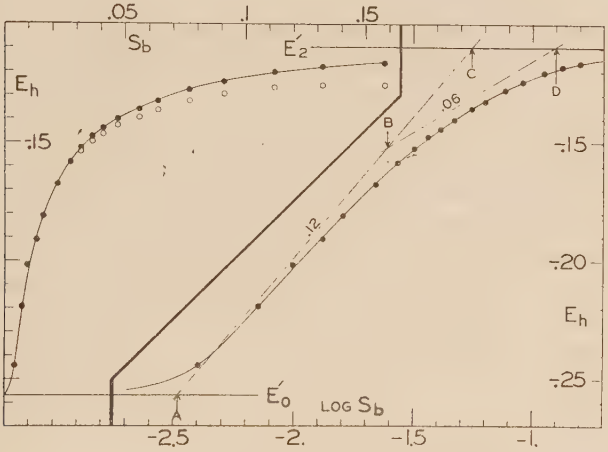


FIG. 2. Iron protoporphyrin IX + nicotine; aqueous solution; 30°. ○ observed; ● corrected for pH change; S (on Fe basis) 2×10^{-4} M; 81 per cent reduced; pH = 11.1; $\mu = 0.35$; aqueous phosphate buffer. The curves are calculated with $\frac{[\mathbf{R}_2][B]^4}{[B_4\mathbf{R}_2]} = K_R$, $\frac{[B_2\mathbf{O}_2][B]^2}{[B_4\mathbf{O}_2]} = K_{O_1}$, $\frac{[\mathbf{O}_2][B]^2}{[B_2\mathbf{O}_2]} = K_{O_2}$, $K_R = 1.2 \times 10^{-10}$, 0.25 log $K_R = -2.48$ (see Point A), $K_{O_2} = 6.05 \times 10^{-4}$, 0.5 log $K_{O_2} = -1.609$ (see Point B), 0.25 log $K_{O_1}K_{O_2} = -0.256$ (see Point C), $K_{O_1} = 1.56 \times 10^{-2}$, 0.5 log $K_{O_1} = -0.903$ (see Point D), $E'_0 = -0.2570$ volt, $E'_2 = -0.1099$ volt.

with the oxidant and reductant. Furthermore, the curvature of the upper part is not as abrupt as would then be expected and it gives clear evidence of tending toward a "0.06 slope" for some distance. These features suggest either $r - q = 2$ or stepwise association involving a $K_O[B]^2$ term as well as a $[B]^4$ term. Under the influence of the last the curve would finally follow a limiting asymptote E_2. To give a fair representation of a distinct possi-

bility the theoretical curve has been drawn with the assumption of the two successive processes:

$$O_2 + 2B \rightleftharpoons B_2O_2$$

$$B_2O_2 + 2B \rightleftharpoons B_4O_2$$

A difficulty ensuing from the last assumption will be noted in the discussion.

Fig. 3 compares the iron mesoporphyrin and iron protoporphyrin systems associating with nicotine in 47.5 per cent alcoholic water solutions. By Proposition III the change of potential shows $K_O > K_R$. Sufficient of the whole course of association is revealed to indicate that K_O is so much greater than K_R that Proposition VI may be applied to the estimate of r. Accordingly $r = 2$. That q is greater than 0 is evident by the departure from the maximal slope as log S_b increases (Proposition VIII). Obviously the data do not extend far enough to decide between $q = 1$ and $q = 2$. The data for the meso system seem to fit the assumption that $q = 1$ and accordingly the theoretical curve has been drawn on that basis but without the intention of declaring a final conclusion. The data for the upper part of the association curve of the proto system are in sufficient disorder to permit either assumption. This is indicated in Fig. 3.

It is evident that very much larger concentrations of associating base would be required to obtain certain proof that the curves of Fig. 3 approach limiting asymptotes having a "0.06 slope." Should this slope be established, it would indicate $q = 1$ by Proposition IX. Nevertheless, a purely mathematical treatment may proceed tentatively on the assumption of $q = 1$ within the limits of the experimental conditions.

Fig. 4 presents a set of data for the association of the iron protoporphyrins with cyanide ion. We may conclude from the data that $K_O > K_R$ but that the ratio of these constants is too low to permit the use of those approximations that facilitate graphical analysis. The data indicate that within the range of cyanide concentration investigated $q = r$. Hogness, Zschiele, Sidwell, and Barron (9) have given spectrophotometric evidence that in this case the oxidant participates in the process

$$O_2(\bar{O}H)_2 + 4\bar{C}N \rightleftharpoons 2O(\bar{C}N)_2 + 2\bar{O}H$$

If this equilibrium be incorporated in the electrode equation, the latter will have a complexity that renders very difficult any

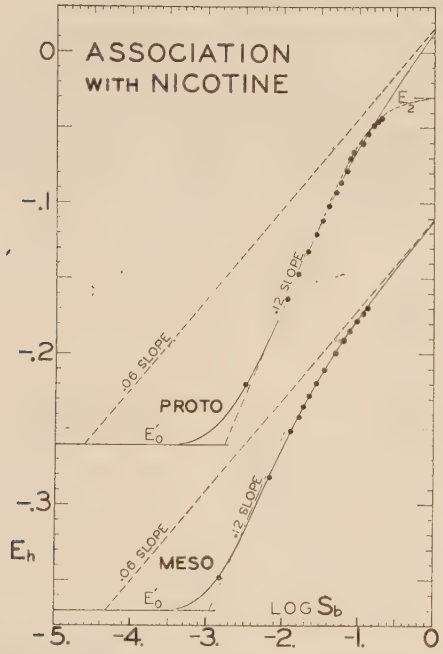


FIG. 3. Relation of potential to log [nicotine]; aqueous alcoholic solution; 30°. The upper curve represents iron protoporphyrin + nicotine in 47.5 per cent ethanol-water solution; phosphate buffer; "pH" = 11.16; $\mu = 0.05$; $S = 1.4 \times 10^{-4}$ M; per cent reduction = 23.5. The curve for $r = 2$, $q = 1$ is drawn with $K_R = 3 \times 10^{-6}$, $K_O = 1.2 \times 10^{-1}$, $E'_0 = -0.2605$ volt; the curve for $r = 2$, $q = 2$ is drawn with $K_R = 3 \times 10^{-6}$, $K_O = 2.2 \times 10^{-2}$, $E'_0 = -0.2605$ volt. The lower curve represents iron mesoporphyrin + nicotine in 47.5 per cent ethanol-water solution; phosphate buffer; "pH" = 11.10; $\mu = 0.2$; $S = 1.4 \times 10^{-4}$ M; per cent reduction = 46. The curve for $r = 2$, $q = 1$ is drawn with $K_R = 1.2 \times 10^{-6}$, $K_O = 2.5 \times 10^{-2}$, $E'_0 = -0.3701$ volt.

graphical analysis. This will be especially true if a similar change in state of aggregation fails to occur in the formation of the cyanide ferroprotoporphyrin. The analysis given in Fig. 4 is based on

the assumption that all four metalloporphyrins are monomeric. It is not maintained, however, that an equally satisfying "fit" may not be possible on some other basis.

Spectrophotometric Measurements of Association—In the study of the coordination of nitrogenous bases with ferro- and ferriporphyrins, the limitations imposed by solubility and other physical properties have forced us to arrive at the characteristics of the equilibria from the consideration of isolated fragments of data. The greater simplicity of solutions containing nitrogenous base and iron porphyrin complexes in but one stage of oxidation would

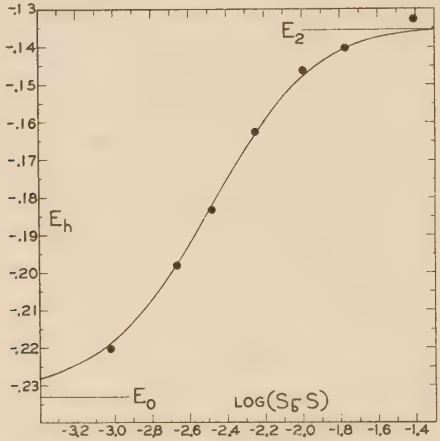


FIG. 4. Iron protoporphyrin + cyanide in phosphate buffer. Relation of potential to log $(S_b - S)$; pH = 11.1; $\mu = 0.4$; initial $S = 2.4 \times 10^{-4}$ M; per cent reduction = *ca.* 13. The curve for $r = 2$, $q = 2$ is drawn with $K_B = 1.5 \times 10^{-6}$, $K_O = 6.39 \times 10^{-5}$, $E'_0 = -0.233$ volt.

appear to make spectrophotometric experiments less ambiguous. And yet the possibility that more than one absorbing species will make invalid the ordinary interpretation and the simpler mathematical analysis of photometric data is disturbing. What proved to be more aggravating in the present instance was the inadequacy of the available instrumental means of determining those slight changes of transmittance of critically important systems that are necessary to the complete elucidation of the equilibria.

The instrument of use was a Bausch and Lomb spectrophotometer. The light housing was replaced by one made according

to the specifications of McNicholas (10). The wave-length drum was calibrated by use of helium and hydrogen Plücker tubes. In the later measurements the absorption tubes were jacketed and surrounded with water circulated from a constant temperature bath.

The following is one item from the spectrophotometric evidence of the reversibility of the association between metalloporphyrin and base. Two ferriprotoporphyrin solutions were prepared with

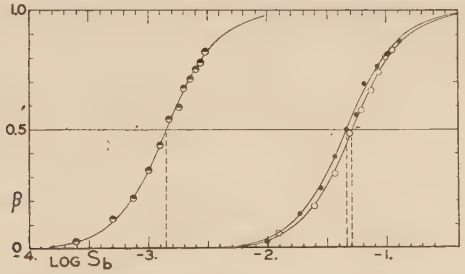


FIG. 5. Spectrophotometric association curves. Ordinate β = [base metalloporphyrin]/S; abscissa, log molar concentration of nicotine. ◓ ferromesoporphyrin-nicotine in 47.5 per cent *ethanol* solution, 0.01 N in NaOH; $S = 6 \times 10^{-6}$ M; $t = 23°$; 10 cm. column; $\lambda = 545$ mμ. The curve is drawn for $\frac{[R_m][B]^2}{[B_2R_m]} = K_R$, $K_R = 2 \times 10^{-6}$, 0.5 log $K_R = -2.85$. ● ferrimesoporphyrin + nicotine in *aqueous* phosphate buffer solution; pH = 10.8; $S = 2.61 \times 10^{-5}$ M; $t = 23°$; 10 cm. column; $\lambda = 580$ mμ. The curve is drawn for $\frac{[O_n][B]^2}{[B_2O_n]} = K_O$, $K_O = 2.13 \times 10^{-3}$, 0.5 log $K_O = -1.34$. ○ ferriprotoporphyrin + nicotine in *aqueous* phosphate buffer solution; pH = 11.1; $S = 6.08 \times 10^{-5}$ M; $t = 23°$; 5 cm. column; $\lambda = 580$ mμ. The curve is drawn for $\frac{[O_n][B]^2}{[B_2O_n]} = K_O$, $K_O = 2.6 \times 10^{-3}$, 0.5 log $K_O = -1.29$.

intermediate concentrations of nicotine such as to give considerable differences in spectral absorption. On dilution of the solution stronger in nicotine to the nicotine concentration of the other and maintenance of the total pigment concentration, the spectral absorptions adjusted to equality.

Paper I (8) describes the procedure for the spectrophotometric study of association.

Fig. 5 shows the data for nicotine ferromesoporphyrin in 47.5

per cent alcohol. The data were transferred to a rectified line by Proposition XIV and the calculated constants were then employed in constructing the type curve for the addition of 2 molecules of nicotine to 1 of ferromesoporphyrin in state of aggregation m. Since the potentiometric data have indicated that $m = n = p = 1$, in aqueous *alcoholic* solution, this is a confirmation of the potentiometric evidence that $r = 2$. The constant is in substantial harmony with $K_R = 3 \times 10^{-6}$, the approximate, graphical estimate on the basis of which the curve of Fig. 3 was constructed.

Fig. 5 also summarizes data for the association of nicotine with ferrimesoporphyrin and ferriprotoporphyrin in *aqueous* phosphate buffer solution. In each case the rectified curves indicated the process $O_n + 2B \rightleftharpoons B_2O_n$ and the type, sigmoid curves have been constructed accordingly. These cases will be discussed later.

Attempts to complete the coordination picture with spectrophotometric studies of ferroprotoporphyrin in *aqueous* solution and ferrimesoporphyrin in *alcoholic* solution with nicotine were unavailing. Reproducible curves in the former case could not be obtained. The readings in solutions of low nicotine content were particularly unstable. The visible spectra of ferrimesoporphyrin and of its nicotine and pyridine complexes in alcoholic solution were too similar to permit measurements of the required precision with the instrument at hand. This was found true for alcoholic solutions of all the ferriporphyrins and their nicotine and pyridine complexes. The complexes of iron hematoporphyrins appeared to be attractive materials for a complete study of the coordination reactions, since the ferrous species were sufficiently soluble and the spectra of the ferric forms were dissimilar enough in aqueous solution, but a light scattering impurity in our preparations prevented significant measurements.

Without dwelling further on such difficulties, we may say that when the spectrophotometric studies are considered in conjunction with the potentiometric association experiments something of interest will appear.

Effect of Ionic Strength—We have preferred to regard the ionic strength effect to be of secondary importance pending the development of other features and provided the variation of the ionic strength of the buffer solutions be not great enough to

affect seriously the study of variables having first order importance. For the preliminary study of the ionic strength effect the pyridine iron mesoporphyrin system in alcoholic solution was chosen. While not ideal, it possesses less unfavorable properties than the others. Fixing the total pyridine, the degree of reduction, and the ratio of buffer salt to buffer acid, we added known quantities of potassium chloride to the system. This will change both the ionic strength and the "pH numbers." A correction for the latter can be made from the known response of the system to changes of "pH." Using the value 0.611 for the dielectric constant obtained from interpolation of Wyman's (11) data for

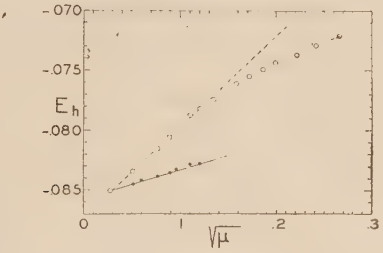


FIG. 6. Relation of ionic strength and potential in the pyridine iron mesoporphyrin system. ○ represents experimental data, ● experimental data at the higher dilutions corrected for pH changes. The slope of the latter curve is 0.0240. Solvent, borate buffer 47.5 per cent in ethanol; initial pH 10.21; total metalloporphyrin 1.4×10^{-4} M; per cent reduction 25.

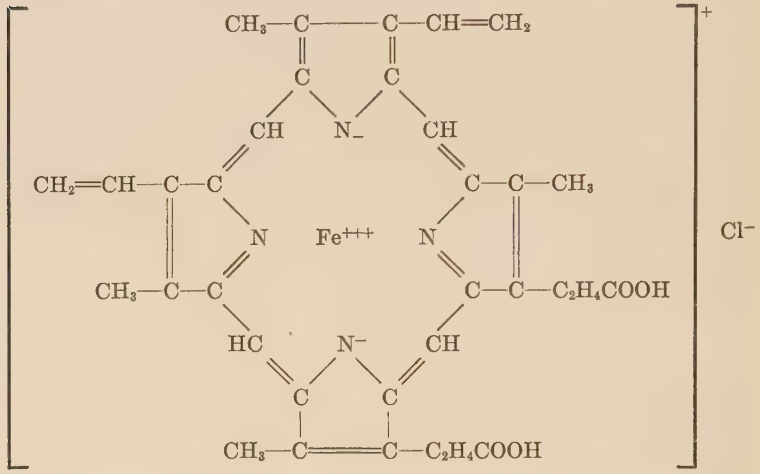
similar water-alcohol mixtures we can deduce from the limiting equation of Debye and Hückel the relation

$$\frac{dE_h}{d\sqrt{\mu}} = 0.0518(z_r^2 - z_o^2)$$

where z_r and z_o are the charges of reductant and oxidant respectively. The observed data are plotted in Fig. 6 together with the same data corrected for the estimated changes of "pH" that accompany the addition of KCl. Assuming that the lower measurements are at ionic strengths low enough to permit their use in guiding the placement of a limiting line, we may make the slope thereof: $dE_h/d\sqrt{\mu} = 0.024$. This corresponds to no integral values of z_r and z_o. Discussion will be postponed.

DISCUSSION

The commonly accepted structure of ferriprotoporphyrin chloride in the solid state is the following.



This is inadequate to account for the behavior of the compound in solution.

Since the evidence is that the oxidant is concerned with a hydroxyl, we may say now that not only ferriprotoporphyrin but also ferrimesoporphyrin and ferrihematoporphyrin appear to associate with 1 hydroxyl ion per iron atom in alkaline aqueous and aqueous alcoholic solution. In the case of dimerization, 2 hydroxyl ions associate per molecule.

It will add to the clarity of a schematic representation if we introduce a postulate, of which we have no evidence; namely, that when there are lacking other associating molecules, the usual coordination number, 6, of iron is satisfied by the entrance of water.[1] Then four coordination positions are occupied by pyrryl nitrogens, one by hydroxyl ion, and one by a water molecule as represented below.[2]

[1] The evidence given by Katz (12) that "*α-Hämine*" and "*β-Hämine*" form no definite hydrates refers to the crystal states as does Richter's (13) evidence of other additions.

[2] The scheme will require modification if data now accumulating should demonstrate that the polymerization noted in several of the ferriporphyrins

$$\left[\begin{array}{ccc} & N^- & \\ & & \overline{OH} \\ N & Fe^{+++} & N \\ H_2O & & \\ & N^- & \end{array}\right]_n + nH^+ + ne \rightleftharpoons \left[\begin{array}{ccc} & N^- & \\ & & H_2O \\ N & Fe^{++} & N \\ H_2O & & \\ & N^- & \end{array}\right]_n$$

or with abbreviated symbols

$$(O\overline{OH})_n + nH^+ + ne \rightleftharpoons R_n$$

The essential points in the following theory are (1) that all associating bases can compete easily with the 2 water molecules for position in the coordination shell of a ferroporphyrin; (2) that neutral associating bases can compete successfully but less easily with the 1 molecule of water in the coordination shell of a ferriporphyrin; (3) that neutral associating bases cannot compete with the hydroxyl ion in the coordination shell of a ferriporphyrin except possibly at very high concentrations of base; (4) that cyanide ions can compete easily with the hydroxyl ion in the coordination shell of a ferriporphyrin.

Let us describe model relations by means of monomeric components and provide a basis for discussion by postulating the following equilibria.

$$\frac{[R][B]^2}{[B_2R]} = K_R \qquad (1)$$

$$\frac{[O][B]^2}{[B_2O]} = K_{O_1} \qquad (2)$$

$$\frac{[O\overline{OH}][H^+]}{[O]} = K_h \qquad (3)$$

$$\frac{[O\overline{OH}][B]}{[BO\overline{OH}]} = K_{O_2} \qquad (4)$$

Note: Process $O\overline{OH} + 2B + H^+ \rightleftharpoons B_2O$ is accounted for by use of Equations 2 and 3.

$$S_r = [R] + [B_2R] \qquad (5)$$

$$S_o = [O] + [O\overline{OH}] + [B_2O] + [BO\overline{OH}] \qquad (6)$$

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is effected through bonds occupying coordination positions of the iron atoms. The spectra of the ferri- and ferroporphyrins in alkaline aqueous solution differ from those in aqueous alcoholic solution. The spectra of the nitrogenous base complexes, however, are unaffected by the change in solvent. Since such changes in spectra usually imply alterations in structure, it is not unreasonable to assume that in alcoholic solution the water of ferri- and ferroporphyrin has been partially or completely replaced by ethanol.

Whence

$$E_h = E_0 + 0.06 \log \frac{S_o}{S_r} + 0.06 \log \frac{K_{O_1} K_{O_2}}{K_R}$$

$$+ 0.06 \log \frac{[H^+](K_R + [B]^2)}{K_{O_1} K_{O_2}([H^+] + K_h) + K_h K_{O_1}[B] + K_{O_2}[B]^2[H^+]} \qquad (7)$$

The cases in which $[H^+]$ and $[B]$ are constant and S_o/S_r is varied have been discussed adequately so that we now may assume a constant degree of reduction. Also the experiments do not cover the direct test of Equation 3 so that we may assume $[H^+] < K_h$. Whence

$$E_h \propto 0.06 \log \frac{[H^+](K_R + [B]^2)}{K_{O_1} K_{O_2} K_h + K_h K_{O_1}[B] + K_{O_2}[B]^2[H^+]} \qquad (8)$$

When [B] is 0

$$\frac{-dE_h}{d\ \mathrm{pH}} = 0.06 \qquad (9)$$

Equation 9 is supported by Barron's data and by the approximate estimates of our own.

In the case of the neutral bases such as pyridine and nicotine we may assume such lack of success in their competition with hydroxyl ion that usually the species B_2O may not form except at very high values of [B]. Whence

$$E_h \propto 0.06 \log \frac{[H^+](K_R + [B]^2)}{K_{O_2} + [B]} \qquad (10)$$

Then, at any constant value of [B]

$$\frac{-dE_b}{d\ \mathrm{pH}} = 0.06 \qquad (11)$$

For confirmation of Equation 11 see Fig. 1. Equation 11 presumably will hold also for the dimeric iron protoporphyrin, since there will obtain an equation comparable to Equation 7 but with the coefficient 0.03 and the term $[H^+]^2$.

At constant degree of reduction and constant $[H^+]$ the final trend of $dE_h/(d \log S_b)$ will depend on whether B_2O or $BO\overline{OH}$ is the final form. If formation of the species B_2O is too difficult, $dE_h/(d \log S_b) = 0.06$ as exhibited.

In the cases of the cyanide systems we may assume such case of competition against $[\overline{OH}]$ that the process will be

$$O\overline{OH} + 2\overline{CN} + H^+ \rightleftharpoons O(\overline{CN})_2$$

involving Equations 2 and 3 as well as Equation 1. Then

$$E_h \propto 0.06 \log \frac{[H^+](K_R + [B]^2)}{K_{O_1} K_h + [B]^2[H^+]}$$

If $[B]^2 > K_{O_1}K_{h_1}/[H^+]$, $\Delta E_h/\Delta$ pH $= 0$. This requires that, when $[H^+]$ is very small, K_{O_1} be very small. While the evidence is that K_{O_1} is small, the final satisfaction of this argument must rest upon a direct estimate of K_{O_1} and this is not available.

There appears a conflict between the *prima facie* evidence of Fig. 2 and that of Fig. 5. The potentiometric evidence that more than 1 nicotine per atom of iron (2 nicotines per mole of dimer in water solution) adds to the oxidant is open to question because of the difficulty of correcting the data at high concentrations of nicotine to a common basis and because it is not certain that more than the estimated number of molecules of base may not add to reductant at very high concentrations of base. The spectral evidence is that 2 molecules of nicotine add per molecule but the spectral evidence will not distinguish the cases $O_2 + 2B \rightleftharpoons B_2O_2$ and $O + 2B \rightleftharpoons B_2O$. The spectral evidence is open to question because of the inherent instrumental and theoretical difficulties.

If the treatment accorded the data of Fig. 2 be correct, and the model of Equation 7 be retained and modified only to fit this case of dimers, the final dominance of the species B_4O_2 would lead to the relation $-dE_b/d$ pH $= 0$. Of this there is no sign. If, however, the species of oxidant containing 4 molecules of nicotine retain 1 hydroxyl ion per iron atom, the relation $-dE_b/d$ pH $=$ 0.06 will be satisfied but the postulated species would violate the usual "rule of 6" for the coordination number of iron.

The data of Fig. 5 on the association of nicotine with ferrimesoporphyrin in alkaline aqueous solution permit two interpretations. Either the metalloporphyrin is monomeric and associates with 2 nicotine molecules in the range of nicotine concentration studied or it is dimeric and adds 1 nicotine molecule

per iron atom. Potentiometric data which would lead to a decision here were unobtainable by reason of the very low solubilities of ferromesoporphyrin and nicotine ferromesoporphyrin already mentioned. The very similar spectral changes observed when nicotine is added to either ferrimesoporphyrin or ferriprotoporphyrin and the close proximity of the two curves of Fig. 5 suggest that the two metalloporphyrins and their complexes with nicotine are highly similar in structure. By this argument ferrimesoporphyrin would be a dimer adding 1 molecule of nicotine per iron atom.

Even a superficial examination of the equations constructed on simplifying assumptions and without the inclusion of possible but unidentified species will show that several important decisions can be made only when consistency is established among the numerical values of several constants. Since the systems so far examined have properties that have precluded the evaluation of some of the constants, there is no point in further discussion at this time. Systems better suited to the examination of doubtful relations should be sought.

What seems to remain clear in addition to the relations developed in the experimental section is that competition between coordinating bases and hydroxyl ions for the bonds of the coordination shell plays a decisive rôle.

Although dimerization of Werner complexes of transition group elements has been observed in several instances (14, 15), the structures proposed for these do not appear applicable to our systems. Our data on the dimerized systems reveal the two coordination centers to be intimately linked energetically. The data of Table V indicate the species in which 1 iron ion is ferric and 1 is ferrous to be quite unstable with respect to the species in which both iron ions are ferric or ferrous. Similarly, there is no evidence in the data of Fig. 5 or of Fig. 2 for the stepwise addition of the first 2 nicotine molecules to the dimer. This interdependence of the iron atoms suggests a hydroxyl bridge between the coordination centers such as Gaines, Hammett, and Walden (15) found in the complexes of iron and phenanthroline. If this holds, we are at a loss to explain the extreme sensitivity of dimerization to apparently trivial alterations in the substitutions on the pyrryl rings. The nicotine iron coproporphyrin system exhibits no polymerization under the same con-

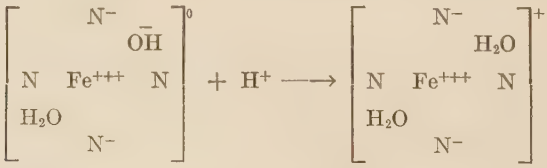
ditions in which the nicotine iron protoporphyrin system is dimeric. Further, Barron (1) reports the pyridine iron protoporphyrin system to be completely dimeric at pH 11.98, whereas the pyridine iron hematoporphyrin system appears completely monomeric at pH 11.75 (Table VI). The well known tendency toward polymerization of compounds carrying aromatic vinyl groups (see for example (16)) suggests the vinyl side chains of protoporphyrin as the site of the dimerizing bond. This conflicts, however, with the interpretation we have given for the spectrophotometric association data on the nicotine ferrimesoporphyrin system. The theory fails to account for the contrasting behavior of pyridine iron protoporphyrin with α-picoline iron protoporphyrin (1).

The evidence for the state of aggregation of the iron porphyrins in the absence of coordinating base is conflicting. The data on Fig. 4 have been analyzed with fair success on the assumption that ferric protoporphyrin is dimeric. This is in accord with the report of Hogness *et al.* (9) on the association of ferriprotoporphyrin with cyanide ion but at odds with our own analysis of the potentiometric data on the system. The theoretical curve of Fig. 2 was constructed on the assumption that both ferro- and ferriprotoporphyrin existed as dimers in alkaline aqueous solution.

If the iron protoporphyrin system in the absence of coordinating base is such that the oxidant is a dimer and the reductant a monomer, the potential should be a unique function of the total concentration of the system. Conant and Tongberg (17) reported the potential to become more negative with increase of the system's concentration, which is the direction predicted on the above assumption. On the other hand Barron reports a change of potential with dilution which is opposite in sign to that observed by Conant and Tongberg and which is greater than would be expected for a monomeric reductant and dimeric oxidant. To complicate matters Barron's data for individual titrations are fairly well described by the equation derived on the assumption that both oxidant and reductant are monomeric. We regret that our own attempts to deal with this system have not yielded reliable data, for the characterization of the metalloporphyrin systems in the absence of coordinating base is essential to a complete understanding of the subject as a whole.

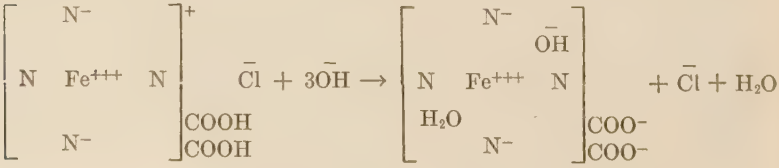
It remains to place Taylor's (3) results with cobalt and manganese mesoporphyrins in harmony with the structural hypothesis here outlined. As Taylor has emphasized, the range of experiment was limited by the properties of the components of his systems. But to be within the realm of the established relations we need only postulate that manganic mesoporphyrin, like the reductants of the iron porphyrins, either does not coordinate with hydroxyl ion or does so only at pH values greater than those used by Taylor. This should remove the pH effect and Taylor found none. The absence of hydroxyl ion should also result in charges leading to a greater ionic strength effect and this is what Taylor found.

The work of other investigators with other methods lends support to the postulated structures. The center of coordination of the postulated compounds bears no net charge. Thus the metalloporphyrins formed from porphyrins having no carboxyl group should be electrically neutral in alkaline solution. Haurowitz (18) reports that the dimethyl ester of ferrimesoporphyrin IX in alkaline alcohol-water mixtures does not migrate in the electric field, while on acidification of the solution the pigment migrates as a cation. This is consistent with the formulation



wherein *sources* of charges are indicated. Haurowitz observed the cyanide compound to migrate as an anion. This also is consistent with our formulation.

To form the disodium salt of ferriprotoporphyrin from the ferriprotoporphyrin chloride our postulate requires 3 equivalents of alkali per gm. atom of iron, as illustrated below.



Morrison and Williams (19) reported titration curves which were complicated by the presence of a solid phase but in which 3 equivalents of alkali are required before the final "break."

In our scheme the oxidant and the reductant of pyridine iron mesoporphyrin bear the same net charges, namely two negative, accounted for by the ions of the carboxyl groups. Therefore, in the simplified theory of Debye and Hückel an "ionic strength effect" of the type noted in the experiment recorded by Fig. 6 should be nil. After correction of the data for pH changes there is a residual effect ascribable to change in ionic strength. It would be interesting to know whether this small residual effect is due to a small proportion of the species B_2O of net charge $z_o = 1\ (-)$. Of course any simple argument in this field is predicated on the assumption that no allowance be made for the spatial distribution of charges and this may play a rôle in molecules of the kind under consideration.

SUMMARY

By potentiometric and spectrophotometric measurements data were obtained for the following.

1 equivalent per iron atom is concerned in the reduction of ferrimesoporphyrin, ferriprotoporphyrin, and ferrihematoporphyrin and in the presence of nicotine, pyridine, α-picoline, or cyanide. Oxidant and reductant of the nicotine iron protoporphyrin system are dimers in aqueous solution, monomers in 47 per cent aqueous alcohol within the range of pH used.

Measurements of the change of potential with increase of concentration of coordinating base, all other conditions being constant, have shown in all the cases that the ferroporphyrins coordinate better with bases than do the ferriporphyrins and that 2 molecules of base per iron atom coordinate with ferroporphyrins. The evidence that coordination occurs with ferriporphyrins is definite but whether 1 or 2 molecules of pyridine, nicotine, or α-picoline coordinate per iron atom is uncertain.

At constant high concentration of associating base and constant degree of reduction $-\Delta E_h/\Delta$ pH = 0.06 for all cases except cyanide, in which case the value is 0.

A structural hypothesis that accounts for the main features is that the ferriporphyrins in alkaline solution are associated with

1 hydroxyl ion per iron atom, that neutral bases compete with difficulty, if at all, with this hydroxyl ion for position in the coordination grouping, while the negatively charged cyanide ions compete successfully.

Owing to limitations imposed by the physical properties of the systems examined, certain quantitative relations flowing from the formulation of this theory have not been determined but their natures have been indicated.

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Vita

The author of this dissertation was born in Baltimore on February 21, 1912. He received his secondary schooling in Tucson, Arizona, and at Woodberry Forest School, Orange, Virginia. He entered The Johns Hopkins University in 1932 and received the Bachelor of Arts degree in 1935. Since that time he has been a student in the Department of Physiological Chemistry, the School of Medicine, where he has pursued the study of physiological chemistry and has undertaken the investigation here described.